สำนักงานวิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

CHAPTER I

INTRODUCTION

Many organic compounds occur in both enantiomeric forms that have identical physical and chemical properties except that they behave differently in a chiral environment. The unique characteristic of enantiomers is most commonly referred to as "chirality".

Chirality is an important aspect in various fields of chemistry and biology due to the fact that the molecular building blocks of natural biological materials are pure enantiomers and they tend to be stereoselective for only one enantiomer in a given pair. For instance, only L-amino acids are nutritional value for animals, L-glutamate is used as a food flavor enhancer, whereas the D-isomers do not have any such properties [1-2].

Currently, chirality is increasingly concerned in pharmacology, agrochemical industry and environmental science. Many chiral drugs and agrochemicals are prepared and supplied as racemates, although the desired activity may be limited to individual enantiopure isomers, as shown in table 1.1. In pharmacology, two enantiomers of a chiral drug may differ significantly in their toxicities, rate of metabolism and selectivity for receptors; therefore, using pure drugs can potentially improve their therapeutic efficiency and selectivity. In 1992, The U.S. Food and Drug Administration issued a guideline for chiral drugs that only active forms could be brought to market, and that each enantiomer of the chiral drugs should be separated before being used [3-4].

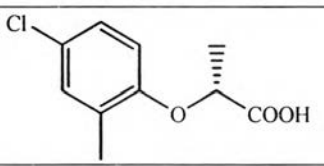
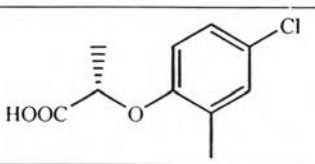
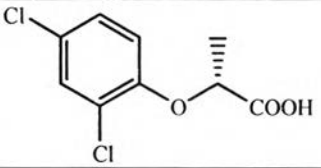
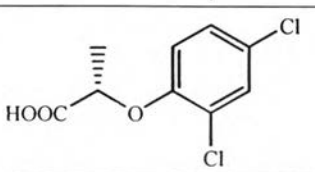
Table 1.1 Different biological activities of enantiomers of some chiral drugs [5].

drug	physiological effect in humans	
	(+)-enantiomer	(-)-enantiomer
barbiturates	excitation	sedation
morphine	minimal effect	strong analgesic
penicillamine	antirheumatic	neurotoxic
thalidomide	mutagenic	sedative-hypnotic

Similarly, in the case of agrochemical products the enantioselective bioactivity is important for herbicides and pesticides containing chiral stereogenic centers. A recent study [6] showed that chiral compounds were accounted for 25% of all agrochemicals in 1995 as compared to 19% in 1980; however, only 7% of total chiral products were sold as single enantiomers. There were evidences that the unwanted isomers of the products showed toxic effects against non-target organism if they were applied as racemate. Furthermore, racemic products also increase the amount of chemical pollutants and provide additional cost in both production and removal of the inactive isomers [6].

Among various classes of chiral herbicides, phenoxypropionates are widely used in cereal crops as weed-killers. Some of these herbicides have been included in the European list of priority pollutants because they present a strong toxicity for the ecosystem and human with carcinogen and endocrine disfunction. Moreover, it has been found that (*R*)-enantiomers of these compound classes are more active than (*S*)-enantiomers and in some cases only (*R*)-enantiomer has a pesticidal activity (table 1.2). For these reasons, several European countries have recently decreed that only the pure (*R*)-enantiomer of phenoxypropionates can be used as herbicides [7-8].

Table 1.2 Structure of some phenoxypropionates that are widely used and marketed [9].

commercial name	structure	
	(*R*)-enantiomer (active form)	(*S*)-enantiomer (inactive form)
mecoprop-methyl		
dichlorprop-methyl		

With increasing awareness of the different bioactivities of enantiomers, it is preferable to use only the purely desired isomer to decrease unwanted side effects and reduce the amount of chemical used. Hence, there are great needs to develop the technology for production, purification and analysis of single enantiomers.

To obtain purely single enantiomers, two alternative approaches can be considered: enantioselective synthesis of the desired enantiomer or separation of pure enantiomers from a racemic mixture. Currently, asymmetric synthesis using chiral auxiliary or catalyst is an outstanding method and it is dynamically developed. Nonetheless, enantiomeric separation techniques are required not only to resolve enantiomers but to examine enantiopurity of chiral reagents, catalysts and products in the asymmetric synthesis as well. For enantiomeric separation on analytical scale, various methods based on chromatographic and electrophoretic techniques have been developed. Both direct and indirect methods were used. Indirect method uses chiral derivatizing agent to form diastereomeric derivatives; therefore, they can be separated by achiral stationary phase. However, the derivatization procedure is tedious and a suitable derivatizing agent in a pure form is sometimes rarely available.
On the other hand, the direct method involves using the chiral selectors either in mobile phase as additive or in stationary phase. Using chiral selectors as additive

requires a greater amount of selectors and there is very little chance of recovery of chiral selectors hence a large amount of selector is wasted [10-11]. In the latter case, chiral selector is chemically bonded or coated on the supported materials; therefore, the chiral selectors and chromatographic column can be used several times.

Gas chromatography (GC) is an accurate and reliable technique for the separation of chiral analytes that can be vaporized without decomposition. Its advantages include reproducibility, sensitivity, limit of detection, speed and simplicity. Derivatized cyclodextrins (CDs) are the most frequently used chiral stationary phases (CSPs) for the direct separation of volatile enantiomers by GC. CDs are available in various forms and they can be derivatized with different functional groups at different positions with various degree of substitution. CDs derivatives are highly selective; especially, for positional isomers and enantiomers over a wide range of organic compounds, such as herbicides and pesticides, environmental pollutants, alcoholic beverages, essential oils, terpenoids, pheromones and phamaceuticals. Most chiral separations achieved on CSPs; however, were based on the accumulated trial-and-error knowledge of the analyst, intuition, and often simply by chance. Neither scheme of choosing the right CSP offers a guarantee for a successful enantiomeric separation because the mechanism of chiral recognition is still not fully understood and a universal model to explain gas chromatographic chiral separation on cyclodextrin-based CSPs is still needed. Separation of enantiomers was thought mostly to be the result of differing degrees of inclusion of the particular analytes into the cavity of cyclodextrin derivatives and the interactions of the analytes with the outer sphere of CDs. Overall, analyte molecules do not interact with the CD exclusively by one mechanism but through a combination of inclusion, hydrogen-bonding, non-polar interactions, dipole-dipole interactions and electrostatic interactions. There are several factors that influence the enantioselectivity, such as the functional groups, substituents and geometry of chiral analytes, type of CD derivatives, substituents and substituted positions on CD rings. Of all contributions to the chiral recognition, the analyte structure seems to be one of the vital factors and the easiest way to vary in a chiral separation system. Nevertheless, only a few studies into the relationship between enantioselectivities of CD derivatives and chiral analytes

were previously carried out [11-18]. Therefore, this research aims to systematically examine the influence of analyte structure on the enantiomeric separation.

Phenoxy acid methyl esters were selected as the analytes of interest because they are widely employed in agriculture as herbicides and mixed into commercial fertilizers to control growth of broadleaf weeds. Furthermore, they are highly toxic pollutant and have slow degradation rate in the environment [1-4]. Phenoxy acid methyl esters with various number and type of substituents at *ortho*-, *meta*-, and *para*-positions were used as chiral analytes. They were enantioseparated by GC using heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)cyclomaltoheptaose (or BSiMe) and heptakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl)cyclomaltoheptaose (or BSiAc) as chiral selectors. Both derivatized β-CDs were separately dissolved in polysiloxane before using as chiral stationary phases. These two phases have been previously used with high success for chiral separation in GC [19-20]. Thermodynamic investigation attained through van't Hoff approach was used to evaluate the interaction between analytes and stationary phase as well as the enantiodifferentiation. Hopefully, the interpretation of the data derived from this research will provide some mechanistic knowledge about the influence of analyte structure on enantioselective selector-analyte binding interaction. This would enhance the possibility of selecting the most appropriate chiral stationary phase and separation condition for the chiral recognition of these phenoxy acid methyl esters, including other analytes having similar structure to the selected compounds.

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